

Decomposition of Lignocellulose by *Cyathus stercoreus* (Schw.) de Toni NRRL 6473, a "White Rot" Fungus from Cattle Dung

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Cyathus stercoreus (Schw.) de Toni NRRL 6473, isolated from aged and fragmented cattle dung collected from a Michigan pasture, effected substantial losses in lignin (45%) from wheat straw during a 62-day fermentation (25°C). The basidiomycete also improved wheat straw digestibility by freeing α -cellulose for enzymatic hydrolysis to glucose (230 mg of glucose per 1,000 mg of fermented residue). The rationale for selecting *C. stercoreus* in attempting to biologically modify the lignin and cellulose components in wheat straw or other gramineous agricultural residues was based on the expectation that this organism is ecologically specialized to enzymatically attack the substructures of native lignins in grasses.

Fungi capable of efficiently degrading the lignocellulosic complex of gramineous agricultural residues to its fractions (e.g., lignin, cellulose, hemicellulose) can be isolated from ruminant dung in grasslands (D. T. Wicklow, R. W. Detroy, and S. Adams, *Mycologia*, in press). In approaching this research, Wicklow et al. (in press) recognized that certain late-appearing fungal colonists probably have evolved enzyme systems specially adapted for attacking the substructures of native lignins and lignocellulose complexes in digested gramineous residues. Central to this reasoning are the facts that natural lignins of grasses are known to differ from those of other plant taxa in the relative frequency of certain chemical substructures (4-6) and undigested lignocellulose from grasses is a principal component of cattle dung in grasslands.

This study examines the ability of *Cyathus stercoreus* (Schw.) de Toni, *Nidulariaceae* (bird's nest fungi), to differentially modify the lignin and cellulose components of wheat straw (WS). *C. stercoreus* grows in and forms basidiocarps on the surface of old, decomposed cow or horse dung and is worldwide in its distribution (1). We isolated the culture by direct transfer of tissue from immature basidiocarps on aged (6 months) cow dung from a pasture near Hickory Corners, Mich. The dung fragments were collected in the fall of 1978.

Straw from Arthur variety wheat was chosen as a substrate for determining the ability of *C. stercoreus* to affect lignin, cellulose, and biomass losses. We also wanted to measure potential increases in digestible carbohydrate. To accom-

plish these objectives, we combined a 62-day, static-culture fermentation of 50 g of WS at 25°C and analytical procedures as described by Wicklow et al. (in press).

Mean losses in biomass, cellulose, and lignin for WS incubated with *C. stercoreus* are given in Table 1. The actual substrate weight loss is probably underestimated because a correction is not included to account for fungal cell mass in the fermented residue. Approximately 20% of the cellulose and 45% of the lignin in WS were degraded through the activities of this basidiomycete. These values differ from those obtained for other coprophilous fungi grown on autoclaved WS. Wicklow et al. (in press) report that an unidentified agaric NRRL 6464 degraded 23% of the lignin, whereas losses brought about by any of the ascomycetes tested never exceeded 4%. Cellulose losses were greater for WS incubated with the unidentified agaric (34%) than with *Cyathus* (20%). Significant to the results of our study, *Cyathus* effected a proportionately greater loss in the lignin as compared with the cellulose component of WS.

C. stercoreus freed five times more α -cellulose for enzymatic hydrolysis to glucose than was available in the unfermented WS control (Table 1). The fermented WS had a greater amount of cellulose per gram (dry weight) than did the unfermented WS control because *C. stercoreus* degraded other components of the WS (e.g., lignin and hemicellulose). Because different amounts of α -cellulose are initially present in the fermented versus unfermented WS, the effectiveness of *C. stercoreus* in increasing the

TABLE 1. Decomposition of WS by *C. stercoreus* and conversion of cellulose in fermented WS to glucose by cellulase^a

Fermentation agent	Wt loss (% dry wt)	Cellulose remaining ^b (%)	Lignin remaining ^c (%)	α -Cellulose (mg) in 1,000 mg of WS ^b	Glucose (mg) from 1,000-mg sample by enzymatic hydrolysis ^d	Conversion (%) of cellulose to glucose ^e
<i>C. stercoreus</i> NRRL 6473	27.6	80.7	55.3	342	230	61
WS control	NA ^f	100	100	306	42	12

^a Calculations were based upon two independent growth experiments with triplicate analysis per 62-day growth experiment. Enzyme was obtained from Miles Laboratories, Elkhart, Ind. Glucose (milligrams) were determined by high-pressure liquid chromatography.

^b Analyses were made by the Goering-Van Soest method for cellulose content (2).

^c Lignin values were determined by the permanganate lignin method (2).

^d Hydrolysis of 1,000 mg of straw sample with 10 IU of cellulase per g of residue for 4 h. One international unit = 1 μ mol produced as glucose from filter paper per min (3).

^e Conversion data were adjusted for weight gain from water addition to glucosyl moiety on hydrolysis. Formula: percent conversion = [(grams of glucose produced) (162/180) (100)]/dry weight of cellulose, grams.

^f NA, Not applicable.

amount of available carbohydrate was obtained by calculating the percentage conversion of the remaining α -cellulose to glucose.

This is apparently the first report in which one of the bird's nest fungi has been examined for its ability to degrade the remains of higher plants (1). Even so, the present findings are hardly surprising given the types of natural substrates from which these fungi are typically recorded. According to Brodie, *Cyathus costatus* Lloyd ex Stevenson and Cash and *Cyathus fimicola* Lloyd have been recorded only on manure from the tropics, whereas other species in this genus are primarily lignicolous, attacking the old stems of shrubs and trees.

Recently, it has been argued that late-appearing basidiomycetes colonizing ruminant dung in grassland ecosystems represent the ecological equivalents of lignin-degrading "white rotting" basidiomycetes from hardwood forests (Wicklow

et al., in press). Our results, based on *C. stercoreus*, provide additional support for this hypothesis.

LITERATURE CITED

1. Brodie, H. J. 1975. The bird's nest fungi. University of Toronto Press, Toronto.
2. Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Handbook 379. U.S. Department of Agriculture, Washington, D.C.
3. Gong, C., M. R. Ladisch, and G. T. Tsao. 1977. Cellulose from *Trichoderma viride*: purification, properties, kinetics, and mechanism. Biotechnol. Bioeng. 19:959-981.
4. Kirk, T. K. 1971. Effects of microorganisms on lignin. Annu. Rev. Phytopathol. 9:185-210.
5. Kirk, T. K., W. J. Connors, R. D. Bleam, W. F. Hackett, and J. G. Zeikus. 1975. Preparation and microbial decomposition of synthetic [¹⁴C]lignins. Proc. Natl. Acad. Sci. U.S.A. 72:2515-2519.
6. Sarkanen, K. V., and C. H. Ludwig (ed.). 1971. Lignins: occurrence, formation, structure, and reactions. Wiley-Interscience, New York.